Author’s response to reviews

Title: GlycA, a marker of protein glycosylation, is related to albuminuria and estimated glomerular filtration rate: the ELSA-Brasil study.

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Editor Comments:

If at all possible, please provide results of GlycA with future risk of kidney function decline, incident CKD/ESRD. Further clarify how your study population was selected. Consider some stratified analyses to better explore GlycA relationship with CKD by patients characteristics.

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Reviewer 1

This is a cross-sectional analysis between GlycA and albuminuria and eGFR in a cohort of 5050 individuals. The associations were modest at best, and the AUC was poor. The ELSA-Brasil study is a longitudinal prospective cohort study with follow-up visits at least 3-4 years after enrollment. Regardless, the participants were enrolled between 2008 and 2010. Thus, the key thing would have been to link GlycA with future risk of renal function decline, CKD, ESRD, etc. The cross-sectional analyses with albuminuria and eGFR do not advance the field. Any new CKD-related biomarker has to add to the information that UACR and eGFR provide. The only value I can see for cross-sectional type analyses is if there was a difference in GlycA by underlying phenotype of CKD despite equal levels of albuminuria or eGFR.

Reviewer 1 points out a limitation of our study, the fact that our study is cross-sectional. We do agree that longitudinal studies, particularly those evaluating the performance of new biomarkers
on clinical events are needed and definitely superior to cross-sectional studies. In the Discussion section, in the paragraph commenting limitations, this aspect is highlighted. Unfortunately, ELSA-Brasil is still a young cohort. We do not have a significant number of renal events (neither cardiovascular) to run powered longitudinal analysis at this moment. We are currently performing the third follow-up visit, but database is not ready. That was the reason why we performed only cross-sectional analysis with baseline data. However, considering that GlycA is a very new biomarker that has never been evaluated specifically in Nephrology, we believe our study adds information to the current literature, by showing an association between GlycA and albuminuria and/or eGFR, suggesting that glycosylation should be further explored and understood in CKD.

Reviewer 2

The manuscript with the topic of GlycA, as a new nuclear mass resonance (NMR) spectroscopy-derived biomarker of protein glycosylation, is related to albuminuria and estimated glomerular filtration rate provided a very interesting and clinically important in prospective study. The hypothesis framed is sure ingesting for the reader. The author had tried to explain the GlycA, as a new nuclear mass resonance (NMR) spectroscopy-derived biomarker and showed there is the association of GlycA with albuminuria and eGFR in the ELSA-Brasil Study. The study is clear with hypothesis, methodology and statistical analysis supporting the results and thus the discussion. The only minor correction is the units mentioned while defining microalbuminuria and macroalbuminuria should be microgram albumin per milligram creatinine instead of 'microalbuminuria as ≥30 mg/g creatinine and macroalbuminuria as ≥300 mg/g creatinine'.

We thank the Reviewer for this correction. We have corrected the unit throughout the text and in Table 1. All these changes are highlighted in red.

Reviewer 3

Interesting analyses. A few issues need to be addressed. 1. How were the 5,050 participants selected out of the total of 15,105 in the cohort selected for this analysis? 2. Use of the terms "microalbuminuria" and "macroalbuminuria" is discouraged by the Kidney Disease: Improving Global Outcomes and other national and international groups. Other terms, such as "moderately increased albuminuria" should be used. 3. A histogram of the distribution of GlycA levels should be provided. 4. Analyses stratified by relevant subgroups (e.g., diabetes) may be quite informative. 5. Continuous analyses of the association between GlycA and albuminuria may be informative, as well. 6. Both the Introduction and Discussion should make a better case for the significance of examining this association between GlycA and albuminuria. What is it about albuminuria that makes this specific measure relevant for an inflammatory marker?
We thank the Reviewer for the review and provide answers to comments and criticisms below.

1. ELSA-Brasil is an ongoing cohort of 15,105 civil servants aged 35 to 74 years from six cities in Brazil. However, GlycA was available only for the Sao Paulo site, comprising 5061 participants. After excluding 11 patients who had missing data on GlycA, a sample of 5050 participants was included in the current analysis. We have clarified this in the manuscript. Considering that there is no major difference in baseline profile among participants recruited in the six sites and the fact that Sao Paulo is the largest sample, we believe that no bias was introduced by the fact that GlycA was measured only among the Sao Paulo participants.

2. We have changed the terms normoalbuminuria, microalbuminuria and macroalbuminuria to categories of albuminuria A1, A2 and A3 throughout the text and tables, thus adopting the nomenclature proposed by KDIGO, as requested by the Reviewer. All changes are highlighted in red.

3. As requested, a histogram was included as Figure 1.

4. We do agree with editor and reviewers that stratified analysis would be interesting to show. However, the reason why we chose to show analyses for the whole population, not stratified for variables such as sex and diabetes, was statistical power. Particularly for the multivariable logistic regression models using albuminuria alone, stratified analysis could lead to overfitting of estimates. Despite the fact that we have a large sample size (n=5050), only 253 participants present albuminuria A2 or A3, being 122 among diabetics (11.6% of diabetic population) and 131 among non-diabetics (3.3% of non-diabetic population). These numbers perform well for the univariable and multivariable models with few variables, but are not sufficient for performing full multivariable models, as the last models presented in our manuscript. By keeping the analyses within the whole population, we could work with 253 diagnosis of albuminuria, a more reliable number for multivariable analyses. HbA1c was entered in all regression models and although, as expected, it caused some attenuation of estimates, models remained significant despite adjustment. If reviewers believe that stratified tables are very much needed we will be pleased to change it, but otherwise we would prefer to keep the presentation of results as they are, showing the effect for the whole population.

5. We agree that continuous models are important to be shown. The relationship between GlycA and albuminuria treated as a continuous variable is shown in Table 2, where linear regression models on albuminuria (log) are depicted. The univariable model is further adjusted for the most important confounding variables, showing that the relationship between GlycA and albuminuria occurs independently of other known cardiovascular risk factors.
6. In the Discussion section, highlighted in red, we have added a comment on possible reasons why glycosilation may be related specifically to albuminuria, trying to still keep clear that these are only hypothesis that could not be tested in the current analysis.

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We thank the Editor and Reviewers for their time in evaluating our manuscript and for the criticisms and suggestions that we believe have improved the manuscript.